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Effect of Ibogaine on the Various Sites of the NMDA Receptor Complex and Sigma Binding Sites in Rat Brain^a

YOSSEF ITZHAK^{b,c} AND SYED F. ALI^d

^c*Department of Biochemistry and Molecular Biology (R-629), University of Miami School of Medicine, P.O. Box 016129, 1600 NW 10th Avenue, Miami, Florida 33101, USA*

^d*Neurochemistry Laboratory, Division of Neurotoxicology, National Center for Toxicological Research, FDA, Jefferson, Arkansas 72079, USA*

ABSTRACT: Although the alkaloid ibogaine is a potent hallucinogenic agent some indications suggest that it may be useful for the treatment of opioid and cocaine addiction. The neurochemical mechanism(s) underlying ibogaine effects remain unclear. In the present study we investigated the interaction of ibogaine with the phencyclidine (PCP) site located in the ionophore of the *N*-methyl-D-aspartate (NMDA) receptor complex, with the NMDA receptor binding site, and with sigma binding sites. In well-washed membrane preparations of rat cortex and cerebellum, the PCP sites were labeled with [³H]MK-801 or [³H]1-[1(2-theinyl)-cyclohexyl]-piperidine ([³H]TCP), and the NMDA receptor with [³H]-CGP 39653. The sigma-1 and sigma-2 binding site in rat cortex and cerebellum were labeled with [³H]pentazocine and [³H]1,3-di-*o*-tolyl-guanidine ([³H]DTG), respectively. Results indicated that ibogaine interacts with high- and low-affinity PCP binding sites in the cortex: $K_i(H) = 0.01\text{--}0.05\text{ }\mu\text{M}$; $K_i(L) = 2\text{--}4\text{ }\mu\text{M}$, and only with low-affinity sites in the cerebellum: $K_i = 2\text{--}4\text{ }\mu\text{M}$. In contrast, ibogaine ($>100\text{ }\mu\text{M}$) had no affinity for [³H]-CGP 39653 binding sites (cortex and cerebellum). The affinity of ibogaine for sigma-1 and -2 binding sites in cortex and cerebellum ranged from 1.5–3 μM . Since NMDA receptor antagonists (*e.g.*, MK-801) are thought to attenuate opioid withdrawal symptoms and cocaine sensitization, it is possible that binding of ibogaine to the PCP sites contributes to its potential 'endabuse' properties. In turn, ibogaine interaction with sigma binding sites may be associated with its adverse effects.

INTRODUCTION

Ibogaine, found in the roots of *Tebernante iboga*, is a potent hallucinogenic indole alkaloid.^{1,2} Anecdotal reports imply that ibogaine reduces craving for various substances of abuse, such as alcohol, opiates and cocaine,³ and animal studies suggest that ibogaine reduces self administration of morphine and cocaine.⁴ Although ibogaine is being investigated, in control studies, as a potential candidate for treatment of substance abuse, the neurochemical mechanism(s) underlying either the hallucinogenic effects of the potential antiabuse properties of this agent remain largely unclear. Several studies have indicated that ibogaine interacts with multiple neurotransmitter systems, such as opioid, serotonin, dopamine and muscarinic receptors, and monoamine transporters.^{5–7} Of particular interest is the interaction of ibogaine with the phencyclidine

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^b Corresponding author. Tel: (305) 243-4635; fax: (305) 243-3955; email: yitzhak@mednet.med.miami.edu

(PCP) binding sites located in the ionophore of the *N*-methyl-D-aspartate (NMDA) type of glutamate receptors.⁸⁻¹⁰ The PCP/NMDA receptor complex is involved with the psychotomimetic effects of PCP and presumably in mechanism(s) that underlie neuroprotection against opiates and cocaine addiction. Since *in vivo* studies suggest that ibogaine is a quite potent agent, it is expected that its affinity for a particular neurotransmitter system could be high (*e.g.*, nanomolar range). However, it appears that the affinity of ibogaine (or its metabolites) for most neurotransmitter systems investigated is relatively low (*e.g.*, micromolar range).

In the present study we investigated the affinity of ibogaine not only for the PCP site, but also the other binding sites associated with the NMDA receptor complex (*e.g.*, the NMDA binding site, glycine and polyamine binding sites). Also the effect of ibogaine on non-NMDA glutamate receptors (*e.g.*, kainate binding sites) and sigma-1 and sigma-2 binding sites in rat cortex and cerebellum was investigated.

MATERIALS AND METHODS

Materials

The following ligands were purchased from New England Nuclear (Boston, MA): [³H]MK-801 (dizocilpine; 56 Ci/mmol), [³H]TCP (1-[1-(2-theinyl)-cyclohexyl]-piperidine; 42 Ci/mmol), [³H]CGP 39653 (2-amino-4-propyl-5-phosphono-3-pentenoic acid; 42 Ci/mmol), [³H]spermidine (21 Ci/mmol), [³H]kainate (32 Ci/mmol), [³H]penta-zocine (56 Ci/mmol) and [³H]DTG (1,3-di-*o*-tolyl-guanidine; 52 Ci/mmol). [³H]Glycine (17.5 Ci/mmol) was purchased from Amersham. Unlabeled drugs were purchased either from Sigma (St. Louis, MO) or Research Biochemical Incorporated (Natick, MA).

Tissue Preparation

For labeling of the binding sites associated with the NMDA receptor complex, and kainate binding sites, tissue was prepared as follows. Cortex and cerebellum of male Sprague-Dawley rats (Charles River, Wilmington, MA) were homogenized in 10 volumes of tris(hydroxymethyl)aminomethane (TRIS)-HCl buffer (50 mM, pH 7.7) and centrifuged at 45,000 × *g* for 15 min (4°C). Pellets were resuspended in 25 volumes of Triton-X 100 (0.04%) and incubated for 20 min at 37°C. An equal volume of TRIS-HCl (100 mM, pH 7.7) was added to adjust the buffer to 50 mM, and the suspension was centrifuged (45,000 × *g*, 20 min). Pellets were then washed 3 times with 50 mM TRIS-HCl buffer (resuspension followed by centrifugation) and finally resuspended in 6 volumes of sucrose (0.32 M). Aliquots were stored at -80°C until used for receptor binding. For the labeling of the sigma binding sites tissue was prepared as described above except that tissue was not treated with Triton-X 100 (for details, see Ref. 11).

Receptor Binding Assays

[³H]MK-801 and [³H]TCP: Tissue aliquots were resuspended in 10 or 15 volumes of TRIS-HCl buffer (5 mM, pH 7.8) containing glutamate and glycine (0.1 mM each) to optimize the binding of the ligand to the PCP site. In a final volume of 0.5 ml, samples containing [³H]MK-801 or [³H]TCP (2 nM each) were incubated (60 min, 25°C) in

the absence and presence of PCP (10 μ M) to determine nonspecific binding, and various concentrations of ibogaine 0.1 to 100,000 nM. [3 H]CGP 39653: Tissue aliquots were resuspended in 10 volumes of TRIS-HCl buffer (50 mM, pH 7.8). In a final volume of 0.5 ml, samples containing [3 H]CGP 39653 (10 nM) were incubated (60 min, 25°C) in the absence and presence of 0.1 mM glutamate to determine nonspecific binding, and various concentrations of ibogaine. [3 H]Glycine: Tissue aliquots were resuspended in 10 volumes of N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES)-KOH buffer (50 mM, pH 7.6) containing 0.1 mM strychnine (to determine binding to the strychnine-insensitive glycine site of the NMDA receptor complex). In a final volume of 0.5 ml, samples containing [3 H]glycine (4.5 nM) were incubated (30 min, 4°C) in the absence and presence of D-serine (0.1 mM) to determine nonspecific binding, and various concentrations of ibogaine. Samples were centrifuged (14,000 $\times$ g, 8 min, 4°C), the pellet was washed with 1 ml cold buffer and dissolved (20% NaOH) before adding liquid scintillation. [3 H]Spermidine: Tissue aliquots were resuspended in 25 volumes of TRIS-HCl buffer (50 mM, pH 7.8) containing glutamate and glycine (0.1 mM each) to optimize the binding to the polyamine site. In a final volume of 0.5 ml, samples containing [3 H]spermidine (3 mM) were incubated (30 min, 25°C) in the absence and presence of spermine (0.1 mM) to determine nonspecific binding, and various concentrations of ibogaine. [3 H]Kainate: Tissue aliquots were resuspended in 10 volumes of TRIS citrate buffer (50 mM, pH 7.4). Samples of 0.5 ml containing [3 H]kainate (10 nM) were incubated (90 min, 4°C) in the absence and presence of glutamate (0.1 mM) to determine nonspecific binding, and various concentrations of ibogaine. [3 H]Pentazocine and [3 H]DTG: Tissue aliquots were resuspended in 10 volumes of TRIS-HCl buffer (5 mM, pH 7.7). Samples of 0.5 ml containing [3 H]pentazocine (2.5 nM) or [3 H]DTG (4 nM) were incubated (60 min, 25°C) in the absence and presence of haloperidol (0.1 mM) to determine nonspecific binding, and various concentrations of ibogaine. Except for [3 H]glycine assays, reaction was stopped by a rapid vacuum filtration through Whatman GF/B filters presoaked in 0.05% polyethyleneimine. Filters were washed (2 $\times$ 4 ml) with the appropriate buffer and radioactivity was determined by scintillation spectroscopy.

Analysis of Receptor Binding Data

Results were analyzed by using the LIGAND curve-fitting program, Version 2.3.10. This weighted, nonlinear model-fitting computer program distinguishes as many as three distinct binding sites. The fit for a specific number of sites was compared using an *F* test incorporated in the program and a *p* < 0.05 to consider a significant better fit for the model tested.

RESULTS

The effect of various concentrations of ibogaine on [3 H]MK-801 and [3 H]TCP binding to the PCP sites within the channel of the NMDA receptor complex in the cortex is illustrated in FIG. 1. Analysis of the inhibition of [3 H]MK-801 binding by ibogaine in the cortex revealed a better fit for a two-site model over a one-site model (*p* = 0.001; *F* = 31.5). The high- and low-affinity constants were calculated as 0.021 and 4.5 μ M, respectively (TABLE 1). Similarly, inhibition of [3 H]TCP binding in the cortex by ibogaine revealed a better fit for a two-site model (*p* = 0.03; *F* = 11.6); the apparent *K_i* values were 0.015 and 3.1 μ M. However, inhibition of [3 H]MK-801 and [3 H]TCP binding

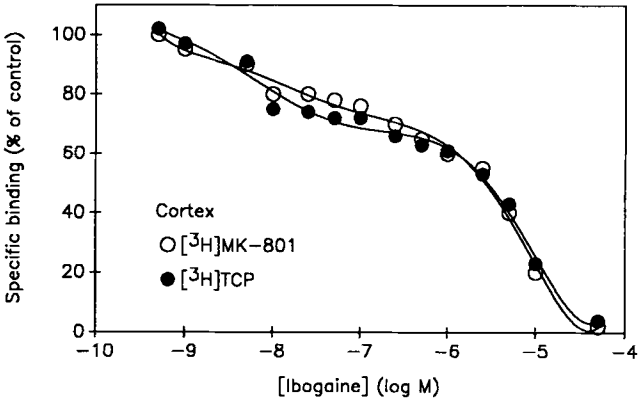


FIGURE 1. Interaction of ibogaine with the PCP binding sites in rat cortex. Assays were carried out in well-washed membrane preparation and in the presence of 100 μ M glutamate and glycine. Analysis of the binding data by the LIGAND program revealed a significant better fit for a two-site model over a one-site model for the inhibition of [3 H]MK-801 binding: K_i (H) = 0.021 ± 0.003 ; K_i (L) = $4.5 \pm 0.6 \mu$ M ($p = 0.001$, $F = 31.5$). For the inhibition of [3 H]TCP binding: K_i (H) = 0.015 ± 0.002 ; K_i (L) = $3.1 \pm 0.2 \mu$ M ($p = 0.03$, $F = 11.6$).

by ibogaine in the cerebellum revealed a better fit for a one-site model with apparent K_i values of 4.1 and 3.5 μ M, respectively (FIG. 2, TABLE 1).

Further investigation of ibogaine effects revealed no interaction with other sites of the NMDA receptor complex. At concentrations up to 0.1 mM, ibogaine had no effect on [3 H]CGP 39653 binding to the NMDA site, [3 H]glycine binding to the strychnine-insensitive glycine site, and [3 H]spermidine binding to the polyamine site. Also, ibogaine had no effect on [3 H]kainate binding to non-NMDA glutamate receptors.

The effects of ibogaine on [3 H]pentazocine binding to the sigma-1 site and [3 H]DTG binding to the sigma-2 site in the cortex and cerebellum are illustrated in FIGS. 3 and

TABLE 1. Effect of Ibogaine on the Binding of Various Ligands to the NMDA Receptor Complex, Kainate and Sigma-1 and Sigma-2 Binding Sites in Rat Cortex and Cerebellum

Ligand	Cortex	K_i (μ M)	Cerebellum
[3 H]MK-801	0.021 ± 0.003 (H)	4.5 ± 0.6 (L) ^a	4.1 ± 0.5
[3 H]TCP	0.015 ± 0.002 (H)	3.1 ± 0.2 (L) ^b	3.5 ± 0.4
[3 H]CGP 39653	N.E.		N.E.
[3 H]Glycine	N.E.		N.E.
[3 H]Spermidine	N.E.		N.E.
[3 H]Kainate	N.E.		N.E.
[3 H]Pentazocine	3.7 ± 0.3		6.6 ± 0.4
[3 H]DTG	1.3 ± 0.2		1.8 ± 0.2

Note: Binding of tritium-labeled ligands was carried out as described in Materials and Methods. Results represent the mean $\pm$ SEM of 3 to 5 experiments. (H) and (L) denote high- and low-affinity sites, respectively. ^a $p = 0.001$ ($F = 31.5$), ^b $p = 0.03$ ($F = 11.6$) for a two-site model. N.E., no effect up to 100 μ M ibogaine.

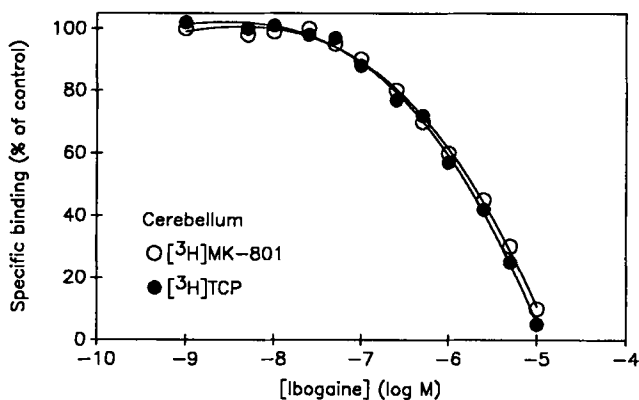


FIGURE 2. Interaction of ibogaine with the PCP binding sites labeled with [³H]MK-801 and [³H]TCP in rat cerebellum. Assays were carried out in well-washed membrane preparation and in the presence of 100 μ M glutamate and glycine. Analysis of the binding data by the LIGAND program revealed a significant better fit for a one-site model.

4, respectively. Analysis of the binding data revealed a better fit for a one-site model. The K_i values of ibogaine for pentazocine and DTG binding sites are summarized in TABLE 1.

DISCUSSION

The major finding of the present study is that ibogaine displayed relatively high affinity (15–21 nM) for the PCP binding sites in the rat cortex. Previous studies have

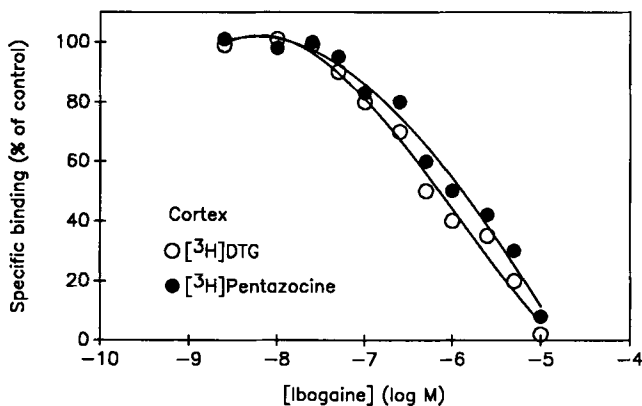


FIGURE 3. Interaction of ibogaine with sigma-1 binding sites labeled with [³H]pentazocine and sigma-2 sites labeled with [³H]DTG in the rat cortex.

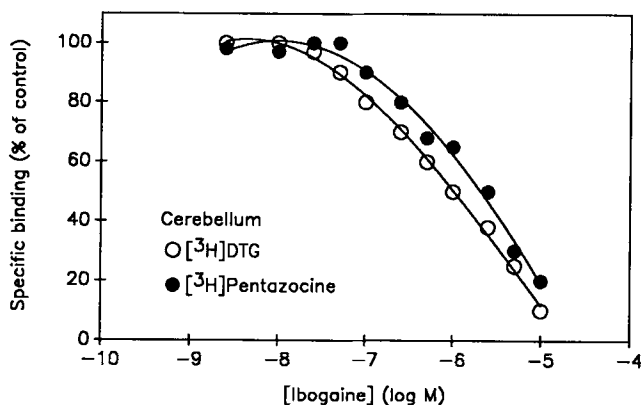


FIGURE 4. Interaction of ibogaine with sigma-1 binding sites labeled with [^3H]pentazocine and sigma-2 sites labeled with [^3H]DTG in the rat cerebellum.

shown that ibogaine interacts with the PCP sites of the NMDA receptor complex, but the affinities observed were in the micromolar range.⁸ However, it appears that ibogaine-sensitive *high-affinity* PCP binding sites were detected only in the cortex, but not in the cerebellum. Differences between the structure of the various subunits of the NMDA receptor in cortex and cerebellum may explain the results observed. The interaction of ibogaine with high-affinity PCP binding sites may be relevant for the reported antiaddictive and hallucinogenic properties of the alkaloid. However, it is unclear whether the interaction with the high-affinity PCP sites is related to the antiaddictive or psychotomimetic effects. There is no distinct dissociation between the binding sites on the NMDA receptor complex that are involved in psychotomimetic/hallucinogenic effects and neuroprotection. Similarly, MK-801 not only attenuates opioid withdrawal¹² and blocks cocaine-induced sensitization,^{13,14} but also produces PCP-like psychotomimetic effects. Thus far, it appears that drugs that bind to the PCP site(s) have not only neuroprotective properties but also hallucinogenic effects. The dissociation between the desirable and undesirable effects produced by PCP-like agents may provide new therapeutics for the management of drug addiction as well as neurodegenerative diseases.

Results of the present study indicate that the interaction of ibogaine with the PCP binding sites within the NMDA receptor complex is rather selective, because the alkaloid had no affinity for other sites of this receptor complex (TABLE 1). These include the NMDA binding site (labeled with [^3H]CGP 39653), the strychnine-insensitive glycine binding site, and the polyamine site (labeled with [^3H]spermidine). In addition, ibogaine had no effect on the non-NMDA glutamate receptors labeled with [^3H]kainate.

The effect of ibogaine on sigma binding sites was investigated because we had previously reported that harmaline—an indole monoamine oxidase inhibitor structurally related to ibogaine but without antiaddictive properties—interacts with sigma-1 and sigma-2 binding sites.¹¹ Results of the present study indicate that the affinities of ibogaine for sigma-1 and sigma-2 binding sites labeled with [^3H]pentazocine and [^3H]DTG, respectively, in the cortex and cerebellum are at the low micromolar range. Similar results were obtained when the effects of ibogaine on sigma binding sites in guinea pig brain and liver were investigated.¹⁵ It appears that the affinities of harmaline and ibogaine for the sigma-1 and -2 binding sites are very similar. Thus, it is unlikely that the

reported antiaddictive properties of ibogaine are related to its interaction with the sigma binding sites.

In summary, the high affinity and specificity of ibogaine for the PCP binding sites, associated with the NMDA receptor complex, may be related to both the antiaddictive and psychotomimetic properties of the alkaloid.

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